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Immunologic Effects of Nickel: I
Suppression of Cellular and Humoral Immunity

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Immunologic Effects of Nickel: I. Suppression of Cellular and Humoral Immunity¹

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The effects of nickel chloride on the cellular and humoral immune responses of mice were studied. A single intramuscular injection of nickel chloride (18.3 mg/kg) caused a significant involution of the thymus within 2 days following treatment. Significant reductions in the *in vitro* mitogen-stimulated response of lymphocytes from nickel chloride-treated mice (24 hr following a single injection of 18.3 or 36.6 mg/kg) were observed for the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A), and the B- and T-cell mitogen pokeweed mitogen (PWM) but not the B-cell mitogen lipopolysaccharide (LPS). Theta-positive but not Ig-positive spleen cells were significantly reduced in nickel-treated mice compared with controls. Significant suppression of the primary antibody response to the T-cell dependent antigen sheep red blood cells was observed following a single injection of 18.3 mg/kg NiCl₂. Natural killer (NK) cell activity was significantly suppressed following a single injection of 18.3 mg/kg NiCl₂. The administration of NiCl₂ (18.3 mg/kg) also decreased the amount of endotoxin required to kill 50% of treated mice, although this was not statistically significant. In all cases the immunosuppressive effects of NiCl₂ were found to be transient with responses returning to normal within a few days. No alteration in the response of mice immunized with the T-cell independent antigen polyvinylpyrrolidone was observed following treatment with nickel. Furthermore, the phagocytic capacity of resident peritoneal macrophages from nickel-treated mice was not significantly different from saline-injected mice. The results indicate that NiCl₂ predominantly affects T-cell mediated immune responses and natural killer cells.

INTRODUCTION

Nickel has been implicated as a potent toxicant in a number of clinically significant pathological findings in man. For example, metallic nickel as inhalable dust, nickel subsulphide, nickel oxide, and nickel carbonyl are known to cause acute pneumonitis, central nervous system disorders, skin disorders such as dermatitis and other hypersensitivity reactions, as well as cancer of the nasal cavity and lungs (see Sunderman, 1977 for a review). In rats, nickel compounds have been shown to be embryotoxic (Sunderman *et al.*, 1978), to produce histopathological alterations in the liver, kidney, testis, and myocardium (Mathur *et al.*, 1977), and to induce sarcomas (Sunderman, 1976).

There is also accumulating evidence for a significant alteration in host defense mechanisms of animals treated with nickel. Waters *et al.* (1975) and Graham *et*

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al. (1975a) reported reduced numbers, viability, and phagocytic and enzymatic activity of alveolar macrophages from rabbits exposed to nickel aerosols. An enhancement of experimental respiratory infections in mice following nickel inhalation (Adkins *et al.*, 1979) and a suppression of the primary antibody response to sheep erythrocytes in mice following exposure to nickel aerosols (Graham *et al.*, 1978) or to parenterally administered nickel (Graham *et al.*, 1975b) have been reported.

This paper deals with the results of studies undertaken to further investigate the effects of nickel, administered parenterally as NiCl_2 , on the functional capacity of the immune system. Immune-function tests were employed which would help to delineate the site of action of nickel on the immune system. These included assays for lymphocyte function (mitogen response, enumeration of T and B lymphocytes, primary antibody response to T-dependent and independent antigens, and natural killer cell activity) and macrophage function (detoxification of endotoxin and phagocytosis).

MATERIALS AND METHODS

Mice. Male CBA/J mice, 9–12 weeks old (Jackson Lab., Bar Harbor, Maine) were used throughout this investigation. The animals were housed in plastic cages on wood shavings and provided food and water *ad libitum*. Mice were held for at least 1 week following shipment prior to use in these studies.

Nickel treatment. A stock-injection solution of nickel chloride (anhydrous, 99% Alfa, Danvers, Mass.) was prepared by dissolving NiCl_2 (20 mg/ml) in saline (pH 7.4). The stock solution was sterilized by filtration using a 0.22- μm sterile filter (Falcon, Oxnard, Calif.). A small amount of green precipitate remained on the filter. The resulting sterile solution was found to contain 18.3 mg NiCl_2 /ml as analyzed by neutron-activation analysis (Dr. J. N. Weaver, Nuclear Engineering Dept., North Carolina State University, Raleigh, N.C.). Dilutions of this stock solution in sterile saline were prepared fresh for each experiment. Unless stated otherwise, mice were given a single intramuscular (im) injection of NiCl_2 in the thigh in a volume not in excess of 0.15 ml. In each experiment control mice were treated identically, except that they were injected with saline.

Mitogen-stimulated lymphocyte response. Mice were killed at various times after treatment and spleens were removed aseptically. Spleen-cell suspensions were obtained as described by Strong *et al.* (1974), and erythrocytes were lysed using NH_4Cl (Boyle, 1968). Cells were washed twice in RPMI-1640 medium, containing 25 mM Hepes (Grand Island Biological Co., Grand Island, N.Y.), L-glutamine, 100 units/ml penicillin, 100 μg /ml streptomycin, and 10% heat-inactivated fetal calf serum, and were adjusted to 5×10^6 viable (i.e., trypan blue excluding) cells/ml. Cells were cultured in sterile flat-bottom microtiter plates (Costar, Cambridge, Mass.). Triplicate cultures containing 5×10^5 cells in 0.2 ml of medium with or without mitogen were prepared for spleen cells from individual treated and control mice. The mitogens used were the following: purified phytohemagglutinin (PHA, Burroughs Wellcome Co., Research Triangle Park, N.C.); concanavalin A (Con A, Miles Lab., Kankakee, Ill.); *Escherichia coli* 0.28:B12 lipopolysaccharide (LPS, Difco Lab., Detroit, Mich.); and pokeweed mitogen

(PWM, Grand Island Biological Co.). Cultures were incubated for 72 hr in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Four hours before harvesting 20 µl of medium containing 0.5 µCi of [methyl-³H]thymidine ([³H]TdR, sp act 6.7 Ci/mm, New England Nuclear, Boston, Mass.) were added to cultures. Cells were harvested with a MASH II automated harvester and the amount of [³H]TdR in DNA was determined using a Model 2650 Tri-Carb liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.). Only optimal mitogen-stimulating concentrations are reported.

Enumeration of T and B lymphocytes. The relative percentage of theta antigen-positive T lymphocytes and Ig-positive B lymphocytes in spleen cell suspensions was determined using a direct immunofluorescent technique. Spleen-cell suspensions were prepared in Hank's balanced salt solution (HBSS, GIBCO) containing 0.5% bovine serum albumin. To these were added either fluorescein-labeled rabbit anti-BALB/C brain serum (anti-theta serum, Litton Bionetics, Kensington, Md.) or goat anti-mouse Ig (Cappel Labs., Cochranville, Pa.). The cells were incubated for 30 min at 4°C, then washed three times in HBSS containing 5% FCS. Slides were prepared, coded, and examined under a Leitz Laborlux 12 microscope with incident-light excitation. At least 200 cells from each preparation were counted and the percentage of fluorescing cells was recorded.

Primary antibody response. The primary antibody response to the T-cell dependent antigen sheep red blood cells (SRBC) was determined using a modification of the IgM antibody-forming plaque assay described by Jerne and Nordin (1963). At various times following a single injection of NiCl₂ mice were immunized with an intraperitoneal injection of 5×10^8 SRBC. Four days after immunization, spleens were removed and the cells producing IgM anti-SRBC antibody were enumerated using the direct plaque-forming cell assay.

The primary response to the T-cell independent antigen polyvinylpyrrolidone (PVP) was determined as described by Lake and Reed (1976). Twenty-four hours following NiCl₂ treatment, mice were immunized with an intravenous injection of PVP (0.125 µg/ml buffered saline, molecular weight 36,000, Sigma, St. Louis, Mo.). Five days later the number of spleen cells producing PVP-specific antibody was determined using a modification of the direct plaque-forming assay of Jerne and Nordin (1963) with PVP-coated SRBC as indicator cells.

Natural killer cell assay. Spleen cells, prepared as above for the mitogen assay, were assayed for natural killer (NK) cell activity using the method described by Kiessling *et al.*, 1977. The *in vitro* subline YAC-1 of the Moloney sarcoma virus-induced YAC lymphoma of mouse strain A (obtained from Dr. Ann Burger, Duke University, Durham, N.C.) was used as the target cell. The YAC-1 cells were grown in stationary suspension cultures in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics (100 units/ml of penicillin and 100 µg/ml streptomycin). The cells were washed once and resuspended to 3×10^6 cells/ml in medium and labeled with ⁵¹Cr (200 µCi ⁵¹Cr as sodium chromate, sp act 200–500 Ci/g, New England Nuclear) for 1 hr at 37°C. After labeling, the YAC-1 cells were washed three times and resuspended to 2×10^5 cells/ml in supplemented medium.

Labeled YAC-1 cells (2×10^4 in a volume of 100 µl) were added to graded

numbers of splenic effector cells (100 μ l) in round-bottom microtiter plates (Titertek/Linbro, Flow Labs, Rockville, Md.). Three replicates of each effector-target cell ratio (25:1, 50:1, and 100:1) were incubated for 4 hr at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. At the end of the incubation, the plates were centrifuged for 5 min at 250g and the supernatant was removed using the Titertek supernatant collection system (Flow Labs). Radioactivity in the supernatant was measured in a gamma counter (Packard Instrument Co.). Spontaneous release of radioactivity was determined by incubating 2×10^4 ⁵¹Cr-YAC-1 cells with 200 μ l of supplemented RPMI medium. Maximum release was determined by adding 50 μ l of 1% Triton X-100 to wells containing 150 μ l of 2×10^4 ⁵¹Cr-YAC-1 cells. Percentage specific ⁵¹Cr release was calculated according to the following formula: $[(E - S)/(T - S)] \times 100$, where E is the ⁵¹Cr released from the target cells in the presence of spleen cells, S is the spontaneous release of ⁵¹Cr, and T is the maximum release of ⁵¹Cr upon addition of Triton X-100.

Endotoxin lethality. Twenty-four hours following a single intramuscular injection of 18.3 mg/kg NiCl₂, mice were injected intraperitoneally with endotoxin (*Salmonella typhimurium* lipopolysaccharide, Difco Labs.) at doses ranging from 25 to 400 μ g/mouse in a volume of 0.2 ml in pyrogen-free saline. A total of six mice per dose was employed. The mortality due to endotoxin shock was scored after 48 hr.

Macrophage phagocytosis. The phagocytic capability of unstimulated resident peritoneal macrophages from mice 24 hr after an injection of 18.3 mg/kg NiCl₂ was determined by the method of Walker and Demus (1975). By using sterile HBSS containing 10 units/ml sodium heparin, resident peritoneal exudate cells (PEC) were washed from the peritoneal cavity of mice. The PEC were washed three times and resuspended to 1×10^6 cells/ml in RPMI supplemented with 10% heat-inactivated gamma-globulin-free fetal calf serum and antibiotics (100 units/ml of penicillin and 100 μ g/ml streptomycin). To each well, in 16-mm diameter 24-well culture clusters (Costar), was added 0.5 ml of PEC suspension. Cultures were incubated at 37°C, 5% CO₂, and 95% air for 2 hr to allow macrophages to adhere. The cultures were washed three times with warm HBSS to remove non-adherent cells and incubated for an additional hour in 0.5 ml supplemented RPMI medium.

Chicken erythrocytes (CRBC) were labeled with ⁵¹Cr by resuspending 0.1 ml of packed CRBC in 200 μ Ci ⁵¹Cr and by incubating the mixture at 37°C for 30 min. The cells were washed three times with HBSS and resuspended to 5×10^6 CRBC/ml. Anti-CRBC antiserum was raised in CBA/J male mice by subcutaneous inoculation of a 20% suspension of CRBC and complete Freund's adjuvant (GIBCO). The animals were reinoculated 14 days later with an intraperitoneal injection of a 10% suspension of CRBC and bled 14 days later. The sera were pooled and heat inactivated as was normal CBA/J serum which was used as a control.

To culture wells containing macrophage monolayers after removal of medium were added 0.5×10^6 ⁵¹Cr-CRBC and a 1:100 dilution of anti-serum or normal serum in a total volume of 0.5 ml/well. At 0, 30, 60, and 120 min the phagocytosis of ⁵¹Cr-CRBC was determined. Culture plates were centrifuged for 5 min at 250g, the supernatant removed, and radioactivity of the samples determined in a gamma

spectrometer (Packard Instrument Co.). Distilled water (0.5 ml) was then added to the culture wells, mixed, and allowed to stand for 5 min at room temperature to lyse CRBC adhering to macrophages. The culture plates were recentrifuged and the supernatant removed and counted. Finally, the cell monolayer was lysed by the addition of 0.5 ml of 0.1 N NaOH and the lysate counted. The macrophage phagocytic activity in each culture was estimated by determining the percentage of total radioactivity associated with the cell monolayer following the water-lysis step:

$$\% \text{ phagocytosis} = \frac{{}^{51}\text{Cr release of NaOH lysate}}{\text{total } {}^{51}\text{Cr release}} \times 100.$$

Upon microscopic examination, cell monolayers were found to contain greater than 90% macrophages based on phagocytized CRBC.

Statistical analysis. Data collected as counts per minute for the mitogen assay were $\log_{10}$ transformed and analyzed statistically using Student's *t* test, Dunnett's *t* test (Dunnett, 1955), or analysis of variance as appropriate. The primary antibody response data were analyzed using Dunnett's *t* test as were the data from the NK and phagocytosis assays. Determination of the 50% lethal dose (LD_{50}) for endotoxin treatment was done using probit analysis. Significant difference between NiCl_2 injected and control mice treated with endotoxin was determined using the relative potency test (Finney, 1971).

TABLE I
THE EFFECT OF NICKEL CHLORIDE ON BODY, THYMUS, AND SPLEEN WEIGHTS

	Days after treatment	NiCl_2 dose (mg/kg)			
		0	9.15	18.3	27.5
Body weight	1	28.5 ± 0.21^a	27.0 ± 0.66	26.6 ± 0.40	26.8 ± 1.02
	2	27.5 ± 0.70	26.0 ± 1.10	24.0 ± 1.20	23.8 ± 0.60^b
	3	27.8 ± 1.30	27.2 ± 1.20	24.9 ± 0.70	24.1 ± 0.80^b
	8	27.7 ± 0.67	27.8 ± 0.50	27.9 ± 0.40	25.2 ± 0.70^b
Thymus/body weight ($\times 10^{-3}$)	1	1.44 ± 0.06	1.50 ± 0.09	1.27 ± 0.06	1.00 ± 0.07^b
	2	1.45 ± 0.08	1.32 ± 0.09	1.16 ± 0.07^b	0.57 ± 0.07^b
	3	1.50 ± 0.08	1.44 ± 0.08	1.05 ± 0.14^b	0.35 ± 0.02^b
	8	1.55 ± 0.06	1.30 ± 0.04^b	1.23 ± 0.05^b	0.66 ± 0.09^b
Spleen/body weight ($\times 10^{-3}$)	1	2.83 ± 0.19	2.86 ± 0.16	2.51 ± 0.18	2.03 ± 0.06^b
	2	2.84 ± 0.20	2.56 ± 0.07	2.55 ± 0.14	1.95 ± 0.08^b
	3	2.73 ± 0.07	2.74 ± 0.20	2.39 ± 0.15	1.84 ± 0.19^b
	8	2.69 ± 0.31	2.73 ± 0.13	3.06 ± 0.06	3.13 ± 0.20

Note. Mice were given a single intramuscular injection of saline (0 mg/kg) or NiCl_2 and the body weight (g) and thymus/body weight and spleen/body weight ratios were determined on Days 1, 2, 3, and 8 postinjection.

^a Mean $\pm$ SE of five mice.

^b $P < 0.05$, Dunnett's *t* test for indicated dosage group on given day post treatment versus saline control.

RESULTS

Lymphoid Organ Weights

Table 1 shows the effect of NiCl_2 on body, thymus, and spleen weight at 1, 2, 3, and 8 days after a single intramuscular injection (9.15, 18.3, or 27.5 mg/kg) of NiCl_2 . At the highest dose, body weight was significantly reduced on 2, 3, and 8 days postinjection and a significant decrease ($P < 0.05$) in the spleen/body weight ratio was observed on Days 1–3 postinjection. Significant decreases ($P < 0.01$) in thymus/body weight ratios were observed on Days 2, 3, and 8 post treatment with 18.3 mg/kg and on Days 1–8 with 27.5 mg/kg.

We examined the possibility that the marked involution of the thymus observed following NiCl_2 treatment might be mediated through corticosterone. Mice were bled from the orbital sinus while under ether anesthesia. The bleeding procedure was limited to less than 2.5 min (i.e., time from removal of mouse from cage to obtaining blood sample) to prevent the stress of handling and ether anesthetization from contributing to the corticosterone levels (Cook *et al.*, 1973). Sera were shipped frozen to Smith Kline Clinical Laboratories (Creve Coeur, Mo.) where corticosterone levels were determined using a radioimmunoassay described by Abraham (1967). Corticosterone levels of 84 ± 44 and 92 ± 33 $\mu\text{g/ml}$ ($\bar{x} \pm \text{SD}$) were measured for saline and nickel injected (18.3 mg/kg) mice, respectively, 24 hr following treatment. There was no significant difference in the levels of this corticoid between the two groups at this time or at 3 and 8 days following treatment.

Mitogen Response

Figure 1 shows the response of splenic lymphocytes from NiCl_2 -injected and saline-injected mice to the T-cell mitogens PHA and Con A and the B-cell mitogen LPS. A highly significant ($P < 0.01$) suppression of lymphocyte responsiveness to PHA and Con A was observed in mice injected with 36.6 mg/kg 24 hr prior to the mitogen-response assay; however, no change was observed in the response to LPS. While significant ($P < 0.05$), suppression of response to PHA and Con A was observed for mice injected with 9.15 and 18.3 mg/kg in this experiment, suppression at these doses was not consistently observed in subsequent experiments.

Table 2 shows the results of two experiments in which the mitogen response of splenic lymphocytes was determined 1, 3, and 8 days following an injection of 18.3 mg/kg. Significant suppression of [^3H]TdR uptake by unstimulated lymphocytes from nickel-injected mice was observed in both experiments at 1 day following treatment and in Experiment 2 at 3 days. Nickel-treated mice had suppressed responses to Con A 1 day following treatment in both experiments (Experiment 1, not statistically significant) and in Experiment 2 at 3 days. The PHA and PWM responses were suppressed in Experiment 1 at 1 day. At 3 days following treatment the responses to LPS and PWM were both enhanced (Experiment 2). No change in mitogen responsiveness was observed at 8 days.

In other experiments groups of mice were injected with either saline or NiCl_2 at 1.8, 3.7, or 7.3 mg/kg 5 days per week for 2 weeks. Three days after the last

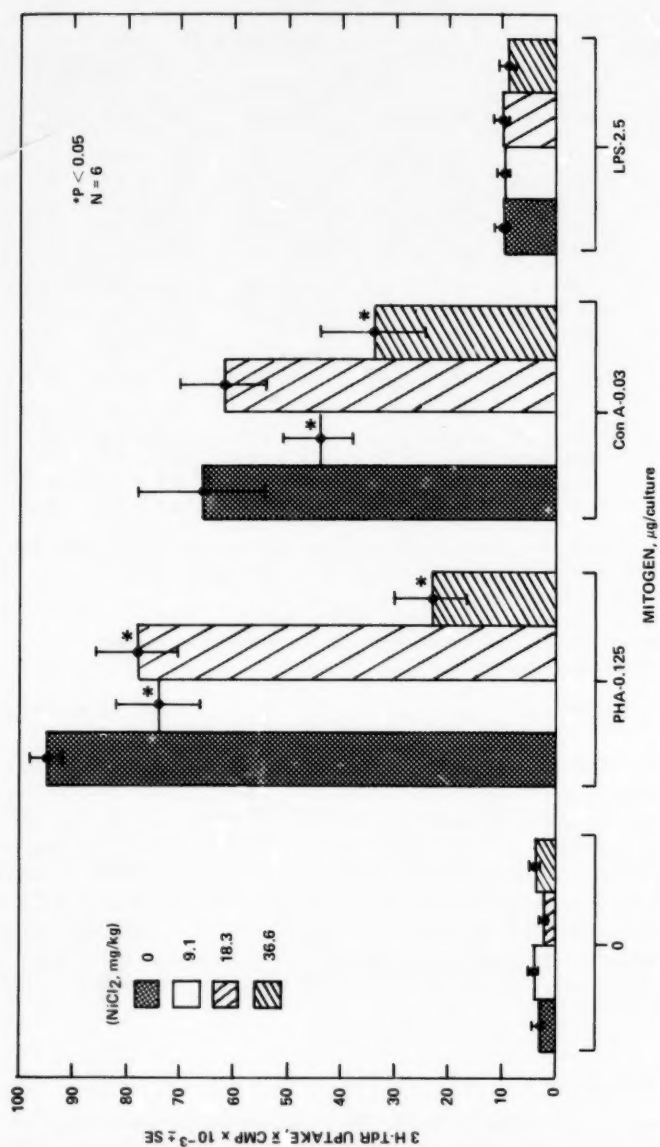


FIG. 1. The effect of nickel chloride on the mitogen-stimulated response of mouse spleen cells ($N = 6$, data analyzed using Dunnett's t test). The mitogen assay was performed 24 hr following a single intramuscular injection.

TABLE 2
SUPPRESSION OF THE MITOGEN-STIMULATED RESPONSE OF MOUSE LYMPHOCYTES
BY NICKEL CHLORIDE

Days after treatment ^b	Mitogen (μg/culture)	Mean cpm × 10 ⁻³ ± SE			
		Exp. No. 1		Exp. No. 2	
		Saline (5) ^a	NiCl ₂ (5)	Saline (10)	NiCl ₂ (10)
1	0	5.1 ± 0.6	2.7 ± 0.7 ^c	11.1 ± 0.8	7.8 ± 0.9 ^c
	PHA—0.125	61.9 ± 7.0	21.5 ± 15.3 ^c	65.8 ± 1.9	59.6 ± 3.6
	ConA—0.03	85.8 ± 6.1	46.9 ± 17.5	79.4 ± 2.7	64.6 ± 5.2 ^c
	LPS—2.5	11.3 ± 0.9	8.1 ± 1.3	22.9 ± 1.1	24.3 ± 2.2
	PWM—1:200 ^d	33.2 ± 1.5	14.9 ± 6.4 ^c	43.9 ± 1.7	44.1 ± 1.5
3	0	8.3 ± 1.3	6.2 ± 0.6	8.3 ± 0.5	5.4 ± 0.5 ^c
	PHA—0.125	90.9 ± 6.5	62.9 ± 12.7	46.2 ± 2.4	48.2 ± 2.2
	ConA—0.03	96.6 ± 5.1	85.5 ± 7.1	52.5 ± 3.1	40.6 ± 3.5 ^c
	LPS—2.5	30.2 ± 3.8	21.0 ± 3.2	18.1 ± 1.2	23.7 ± 1.2 ^c
	PWM—1:200	34.3 ± 3.9	32.1 ± 2.6	33.0 ± 2.2	42.4 ± 1.8 ^c
8	0	6.8 ± 0.7	5.9 ± 1.2	5.2 ± 0.6	3.9 ± 0.5
	PHA—0.125	87.4 ± 7.7	74.9 ± 8.7	51.5 ± 7.1	50.1 ± 8.4
	ConA—0.03	99.0 ± 10.4	81.4 ± 10.7	51.9 ± 5.5	55.4 ± 8.4
	LPS—2.5	25.5 ± 2.5	25.2 ± 1.9	18.3 ± 1.2	16.4 ± 1.1
	PWM—1:200	34.4 ± 1.3	41.7 ± 5.8	35.8 ± 0.7	36.9 ± 2.0

Note. Mice were injected with saline (0 mg/kg) or nickel chloride at the indicated doses. The mitogen response data were analyzed using Student's *t* test with * signifying $P < 0.05$.

^a Number of mice per group.

^b Single intramuscular injection of 18.3 mg/kg NiCl₂ or saline.

^c $P < 0.05$, Student's *t* test, saline versus NiCl₂-injected mice for each mitogen.

^d Dilution of pokeweed mitogen.

injection body, thymus and spleen weights were measured and the mitogen-stimulated response of spleen cells was determined. Table 3 shows the results of the mitogen assay. A significant decrease in [³H]TdR uptake by unstimulated lymphocytes was observed at each nickel dose compared with the saline control. Significantly greater responses were observed for Con A or PWM at the highest dose and for LPS at all doses. The thymus-to-body-weight ratio of mice repeatedly injected with NiCl₂ at all doses was significantly ($P < 0.01$) decreased compared with saline controls (data not shown). No differences in the body weight or spleen-to-body weight ratio were observed between treated and control mice at any NiCl₂ dose.

T and B Lymphocytes

Table 4 shows the effect of NiCl₂ (18.3 mg/kg) on the percentage of theta-positive (T) and Ig-positive (B) spleen cells 24 hr after injection. A significant ($P < 0.05$) decrease in the percentage of T lymphocytes was observed in the spleens of treated mice, but no difference in B lymphocytes was observed.

TABLE 3
THE EFFECT OF REPEATED EXPOSURE TO NICKEL CHLORIDE ON THE MITOGEN-STIMULATED LYMPHOCYTE RESPONSE

NiCl ₂ dose ^a (mg/kg)	Mean cpm $\times 10^{-3} \pm$ SE				
	0	Mitogen (μ g/culture)			
		PHA—0.125	ConA—0.03	LPS—2.5	PWM—1:200
0	8.7 $\pm$ 0.5	64.7 $\pm$ 6.2	56.2 $\pm$ 5.2	12.0 $\pm$ 0.9	30.9 $\pm$ 3.5
1.8	5.4 $\pm$ 0.9 ^b	65.0 $\pm$ 4.4	65.7 $\pm$ 4.1	17.8 $\pm$ 1.8 ^b	35.8 $\pm$ 1.2
3.7	6.0 $\pm$ 0.9 ^b	67.6 $\pm$ 4.5	64.3 $\pm$ 3.8	17.6 $\pm$ 1.9 ^b	39.5 $\pm$ 1.2
7.3	5.0 $\pm$ 0.7 ^b	69.4 $\pm$ 2.0	72.3 $\pm$ 4.8 ^b	20.8 $\pm$ 1.3 ^b	41.3 $\pm$ 2.8 ^b

^a Mice injected intramuscularly 5 days per week for 2 weeks. Eight mice per treatment group.

^b $P < 0.05$, Dunnett's t test, compared to saline-injected controls (0 mg/kg).

Primary Antibody Response

Table 5 shows the effect on the primary antibody response (T-cell dependent) to SRBC. A significant suppression ($P < 0.05$) in PFC/10⁶ cells and PFC/spleen was observed for mice injected with 18.3 and 27.5 mg/kg 24 hr prior to immunization with SRBC. Figure 2 shows the effect of a single injection of 18.3 mg/kg NiCl₂ on 1, 3, or 8 days prior to immunization with SRBCs. The number of PFC/10⁶ cells was significantly ($P < 0.05$) suppressed on Day 1 post treatment, and the PFC/spleen was suppressed ($P < 0.05$) on Days 1 and 3 post treatment. No significant difference in response (T-cell independent) to polyvinylpyrrolidone was observed for treated mice (4.6–18.3 mg/kg) 24 hr prior to immunization with PVP (Table 6).

Natural Killer Cell Activity

Figure 3 shows the effect on splenic natural killer cell activity. Significant ($P < 0.05$) suppression of NK activity was observed in mice injected 24 hr previously with 18.3 and 36.6 mg/kg, but not at 9.15 mg/kg. Significant ($P < 0.05$) suppression of NK activity following a single im injection (18.3 mg/kg) persists for only 24 hr (Table 7). However, when mice were given a single intraperitoneal injection (18.3 mg/kg) suppression of NK activity persists for at least 48 hr (Table 8).

TABLE 4
THE EFFECT OF NICKEL CHLORIDE ON THE PERCENTAGE OF T AND B SPLENIC LYMPHOCYTES 24 hr FOLLOWING A SINGLE INJECTION

Treatment	Mean $\pm$ SE	
	B lymphocytes	T lymphocytes
Saline (6) ^a	40.3 $\pm$ 1.4	27.0 $\pm$ 1.7
NiCl ₂ (6) ^b	41.1 $\pm$ 2.2	19.0 $\pm$ 2.1 ^c

^a Number of animals tested.

^b Single intramuscular injection of 18.3 mg/kg NiCl₂.

^c $P < 0.05$, Student's t test.

TABLE 5
SUPPRESSION OF THE PRIMARY ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS
BY NICKEL CHLORIDE

NiCl ₂ dose (mg/kg)	Mean $\pm$ SE	
	PFC ^b /10 ⁶ cells	PFC $\times$ 10 ⁻⁴ /spleen
0 ^a	532 $\pm$ 71	8.95 $\pm$ 1.67
4.6	397 $\pm$ 89	6.72 $\pm$ 1.89
9.15	564 $\pm$ 119	7.13 $\pm$ 2.40
18.3	184 $\pm$ 39 ^c	2.25 $\pm$ 0.51 ^c
27.5	91 $\pm$ 28 ^c	1.06 $\pm$ 0.37 ^c

^a Six mice per NiCl₂ dose, all mice injected with SRBC 24 hr following nickel injection.

^b Plaque-forming cells.

^c $P < 0.05$, Dunnett's t test, compared to saline-injected controls (0 mg/kg).

Endotoxin Lethality

The LD₅₀ dose for endotoxin from *Salmonella Typhimurium* in saline-injected mice was found to be 266 μ g/mouse or 8.9 mg/kg. For mice injected with 18.3 mg/kg NiCl₂ 24 hr prior to endotoxin, the LD₅₀ dose was 186 μ g endotoxin/mouse or 6.2 mg/kg. While there was a decrease in the dose of endotoxin required to kill 50% of mice injected with NiCl₂, this decrease was not significantly different as determined by the relative potency test (Finney, 1971).

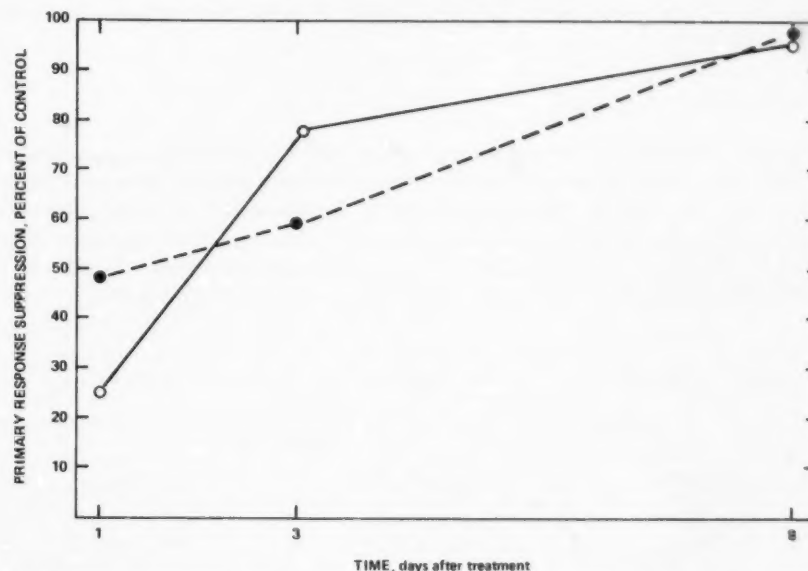


FIG. 2. Suppression of the primary antibody response to sheep red blood cells by nickel chloride (PFC/10⁶ cells, O; PFC/spleen, ●; $N = 6$, data analyzed using Dunnett's t test).

TABLE 6
THE EFFECT OF NICKEL CHLORIDE ON THE PRIMARY ANTIBODY RESPONSE TO POLYVINYLPYRROLIDONE

NiCl ₂ dose (mg/kg)	Mean $\pm$ SE	
	PFC/10 ⁶ cells	PFC $\times$ 10 ⁻³ /spleen
0 ^a	27 $\pm$ 3	2.86 $\pm$ 0.39
4.6	18 $\pm$ 5	2.17 $\pm$ 0.65
9.15	19 $\pm$ 7	2.44 $\pm$ 0.95
18.3	30 $\pm$ 4	3.19 $\pm$ 0.53

^a Six mice per NiCl₂ dose, all mice injected with polyvinylpyrrolidone.

Macrophage Phagocytosis

Figure 4 shows the phagocytic activity of resident peritoneal macrophages 24 hr following a single injection of NiCl₂ (18.3 mg/kg). Each datum represents the mean of triplicate cultures of macrophages pooled from 10 saline-injected or 10 NiCl₂-injected mice. No difference was observed in the phagocytic activity of macrophages from nickel-treated mice for CRBC compared with saline controls in the presence or absence of antibodies to CRBC.

DISCUSSION

A single intramuscular injection of NiCl₂ (18.3 mg/kg) caused a significant involution of the thymus 2 days following treatment. Thymic involution was not

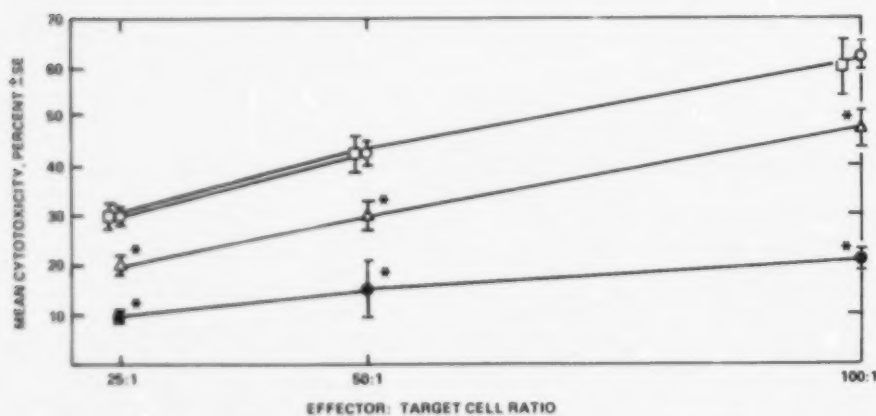


FIG. 3. The effect of nickel chloride on natural killer cell activity. Groups of six mice were given a single intramuscular injection of saline, ○; 9.15 mg/kg NiCl₂, □; 18.3 mg/kg NiCl₂, △; or 36.6 mg/kg NiCl₂, ●. Natural killer cell activity was assayed 24 hr later. Data were analyzed using Dunnett's *t* test with * signifying *P* < 0.05.

TABLE 7
THE EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF NICKEL CHLORIDE ON NATURAL KILLER CELL ACTIVITY

Days after treatment ^a	% Specific lysis ($\bar{x} \pm SE$)		
	25:1 ^c	50:1	100:1
Control (9) ^b	29.0 $\pm$ 2.0	40.6 $\pm$ 2.2	48.6 $\pm$ 2.2
1 (5)	18.6 $\pm$ 1.0 ^d	30.1 $\pm$ 1.0 ^d	39.1 $\pm$ 0.9 ^d
2 (5)	23.5 $\pm$ 2.9	31.6 $\pm$ 2.6 ^d	38.8 $\pm$ 2.8
3 (5)	24.0 $\pm$ 2.8	33.6 $\pm$ 3.0	42.7 $\pm$ 2.8

^a Mice injected im with 18.3 mg/kg NiCl₂, controls injected with saline.

^b Numbers in parentheses are numbers of mice treated. Three groups of three mice were each injected with saline on Days 1, 2, and 3, analysis showed no difference among the three groups and so data from these animals were combined.

^c Effector: target-cell ratio.

^d $P < 0.05$, Dunnett's *t* test, compared with pooled controls.

associated with an increase in serum corticosterone levels. A significant decrease in the percentage of theta-positive, but not Ig-positive B lymphocytes, was observed in the spleens of mice injected with nickel chloride 1 day prior to examination. Significant reductions in the lymphoproliferative response were observed for the T-lymphocyte mitogens PHA and Con A, but not the B-lymphocyte mitogen LPS 24 hr following a single injection of NiCl₂. Enhanced responses to LPS and PWM were observed 3 days following a single injection and 1 day following multiple injections of NiCl₂. The enhancement of lymphocyte responses to B-cell mitogens may be a consequence of enrichment of B lymphocytes in the spleen due to depletion of T lymphocytes. Suppression of the primary antibody

TABLE 8
THE EFFECT OF A SINGLE INTRAPERITONEAL INJECTION OF NICKEL CHLORIDE ON NATURAL KILLER CELL ACTIVITY

Days after treatment ^a	% specific Lysis ($\bar{x} \pm SE$)		
	25:1 ^c	50:1	100:1
Control (9) ^b	16.9 $\pm$ 1.0	27.8 $\pm$ 1.4	34.9 $\pm$ 1.3
1 (5)	7.9 $\pm$ 2.2 ^d	13.4 $\pm$ 2.8 ^d	19.3 $\pm$ 3.5 ^d
2 (5)	7.0 $\pm$ 0.3 ^d	12.0 $\pm$ 0.7 ^d	14.3 $\pm$ 1.1 ^d
3 (5)	13.3 $\pm$ 2.4	21.0 $\pm$ 3.2	23.3 $\pm$ 4.1 ^d

^a Mice injected i.p. with 18.3 mg/kg NiCl₂, controls injected with saline.

^b Numbers in parentheses are numbers of mice tested. Three groups of three mice were each injected with saline on Days 1, 2, and 3, analysis showed no difference among the three groups and so data from these animals were combined.

^c Effector: target-cell ratio.

^d $P < 0.05$, Dunnett's *t* test, compared with pooled controls.

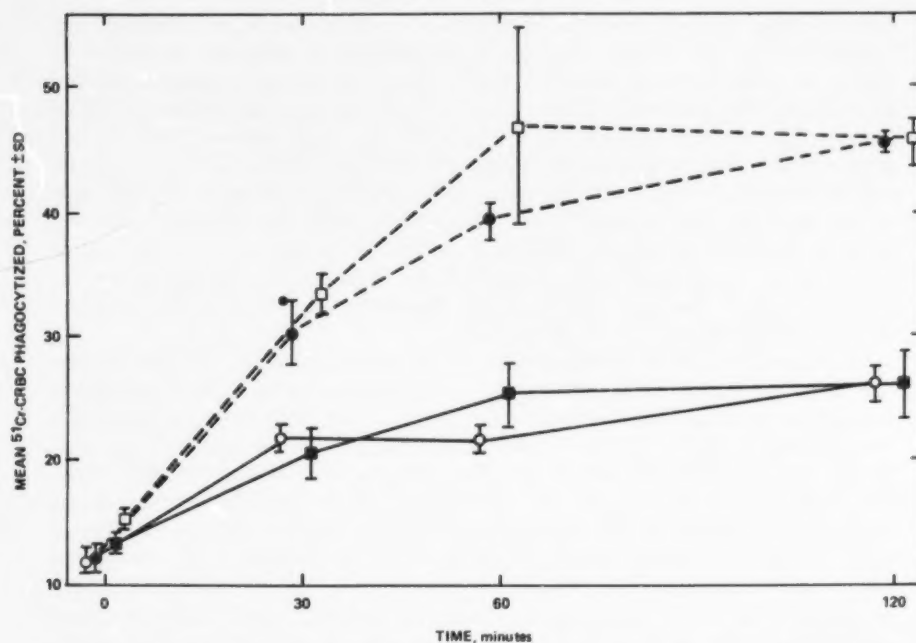


FIG. 4. The effect of nickel chloride on macrophage phagocytosis. Resident peritoneal macrophages from saline-injected mice were cultured with CRBC in normal mouse serum (○) or mouse anti-CRBC serum (□). Macrophages from NiCl_2 -injected mice were cultured with CRBC in normal (■) or anti-CRBC (●) serum.

response to the T-cell dependent antigen sheep red blood cells, but not the T-cell independent antigen polyvinylpyrrolidone was observed following a single injection of NiCl_2 . These effects were reversible with responses returning to normal by 8 days following treatment.

Nickel chloride also suppressed natural killer cell activity. In mice given a single injection of NiCl_2 , natural killer cell activity was significantly reduced. Macrophage phagocytosis was not affected by nickel; however, treated mice were more susceptible to the lethality of endotoxin. The results of this study strongly suggest that nickel chloride given as a single injection acts predominantly on T lymphocytes and natural killer cells.

These results confirm and extend the work of others on the immunosuppressive effects of nickel. Nickel has been found to depress circulating antibody titers to T_1 phage (Figoni and Treagan, 1975); to suppress the primary antibody response to sheep erythrocytes (Graham *et al.*, 1975b); to inhibit the interferon response of cells treated with nickel *in vitro* (Treagan and Furst, 1970); and to increase the susceptibility to pulmonary infections following inhalation (Adkins *et al.*, 1979).

The mechanism(s) by which nickel causes these effects is unknown. Nickel is known to be cytotoxic for alveolar macrophages *in vitro* (Waters *et al.*, 1975). Our results suggest that T lymphocytes and natural killer cells are more suscep-

tible to nickel toxicity than are B lymphocytes or macrophages. Whether this toxicity is direct or indirect remains to be determined, although our failure to detect increases in serum corticosterone levels argues against a steroid-mediated cytotoxicity. The observed effects may, however, be mediated by the endocrine system. The acute administration of Ni(II) salts has been reported to cause hyperglycemia and hyperglucagonemia followed by hyperinsulinemia in rats (Horak and Sunderman, 1975a, b; Horak *et al.*, 1978). We found that mice injected with nickel chloride were hyperglycemic within 24 hr following injection (data not shown), as determined by the presence of glucose in their urine using reagent test strips (Labstix, Ames Co., Elkhart, Ind.). Whether the hyperglycemia, induced by nickel, has any causal relationship with the observed effects on the immune system remains to be determined.

Of particular interest are the results of the effects of nickel on natural killer cell activity. Natural killer cells have been implicated as an important effector mechanism in tumor-cell cytotoxicity (Herberman *et al.*, 1979). NK cells display spontaneous *in vitro* and *in vivo* cytotoxicity against certain syngeneic and allogeneic tumor cells in the absence of immunological priming and therefore, have been suggested to play a significant role as one of the first lines of defense against newly arising malignant cells (Herberman, 1981). In light of the known carcinogenic potential of nickel compounds (Sunderman, 1981; Stoner *et al.*, 1976), it is interesting to speculate as to whether suppression of NK cell activity by nickel as reported here is in any way related to the observed nickel-induced tumor development. For example, it has been shown that mice of the A strain, which have low NK cell activity (Herberman *et al.*, 1979), exhibit a significant increase in the average number of lung tumors following exposure to nickelous acetate (Stoner *et al.*, 1976). A-strain mice are also susceptible to the induction of lung tumors by urethane, which also depresses the already low NK cell activity of these mice (Gorelik and Herberman, 1981). Whether the development of tumors in A-strain mice following nickel treatment is in some way related to lowered NK cell activity as may be true with urethane is an intriguing question which remains to be answered.

In light of the present results, further elucidation of the mechanism(s) of immunosuppression by nickel is warranted. In particular the effects of nickel on NK cell activity and the possible relationship between suppression of these cells and the known carcinogenesis of nickel compounds merit further investigation.

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